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Composition Studies on Tobacco XXXVIII

Evidence for the Origin of the High Molecular Weight, Acidic Pigment in Cigarette Smoke Condensate*

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Cigarette smoke condensate contains significant amounts of high molecular weight pigments that superficially resemble the brown pigments of tobacco leaf (1). On the basis of solubility, the condensate pigments can be separated into acidic and basic substances (2) and the former can be further divided into weakly and strongly acidic subfractions. Initial work on the total acidic pigment from smoke condensate showed the isolated material to be heterogeneous with major, minor and trace components having molecular weights of about $\geqslant$ 100,000, 4,000 and 10,000 — $\geqslant$ 100,000 respectively (3). The unfractionated acidic pigment contained iron, chlorogenic acid and a series of amino acids but most of the material was refractory to hydrolysis and alkaline fusion. Later work was conducted on the weakly acidic and strongly acidic subfractions of the condensate pigment (4, 5). Most of the weakly acidic subfraction was found to be nondialyzable and to contain a component having a molecular weight of $\geqslant$ 100,000, which yielded a silicone, nicotine and a series of bases on alkaline fusion (4). This represented the first report of nicotine bound in a high molecular weight structure in smoke condensate. Subsequently, the strongly acidic subfraction of the acidic condensate pigment was shown to be qualitatively similar to the weakly acidic subfraction (5). Most recently, the nondialyzable weakly acidic subfraction was also found to contain significant amounts of fatty acids and bases (6) released by saponification and it is probable that the strongly acidic subfraction also contains such moieties.

Early work on the brown pigments of tobacco leaf showed the presence of major components in the $<$ 3,000–40,000 molecular weight range (1, 7) and of chlorogenic acid, amino acids, iron and, in some subfractions, rutin in the hydrolytic products (8, 9). Following the demonstration of nicotine and related bases in the condensate pigment, a similar search for these components in the alkaline-soluble leaf pigment yielded positive results (10). Subsequently, hydrolyzable volatile and nonvolatile fatty acids were also found in the alkaline-soluble leaf pigment (6), thus confirming the close similarity between the leaf and smoke constituents.

This similarity has led to a postulation that the leaf pigment is a precursor of the condensate pigment in the smoking process (1). According to this conception, the sharp thermal gradient behind the burning cone of a cigarette causes eruption of cells adjacent to the approaching cone. In the eruption, cell contents, including leaf pigment, are expelled into the aerosol stream and the pigment may be partially degraded. The leaf pigment or degradation products thereof then react further with smoke components to increase the overall molecular weight and produce the small structural differences observed between leaf and condensate pigments.

Two factors mitigate against the validity of this postulation. First, no evidence exists that cellular eruption occurs in a burning cigarette although the presence of a large number of metallic elements in the smoke (1) may be an indication of such a process; the occurrence of heat-labile steroidal glycosides in smoke has been explained on the basis of dry distillation rather than cellular eruption (11). The second factor is that the condensate pigments may not be true smoke components but may be artifacts produced in the isolation procedure, since extremes of pH are employed, or by reactions of smoke constituents in the smoke collection apparatus in a manner similar to the nitrosamines (12), nitrophenols (13) and possibly cyanohydrins (14). Because of the close similarity of leaf and smoke pigments, this latter possibility requires either a highly fortuitous set of qualitative and quantitative circumstances during isolation and/or smoke collection or acceptance of the fact that the leaf pigments may also be artifacts produced in the isolation procedure from tobacco. The isolation of leaf pigments under comparatively mild conditions makes the latter possibility somewhat improbable (8, 9).

The question of the origin of the condensate pigments is more than an academic problem since these components may play some role in the biological activity of smoke condensate. Recent studies have shown that the acidic condensate pigments are not tumor-promoting when tested by topical or parenteral routes in mice (15).

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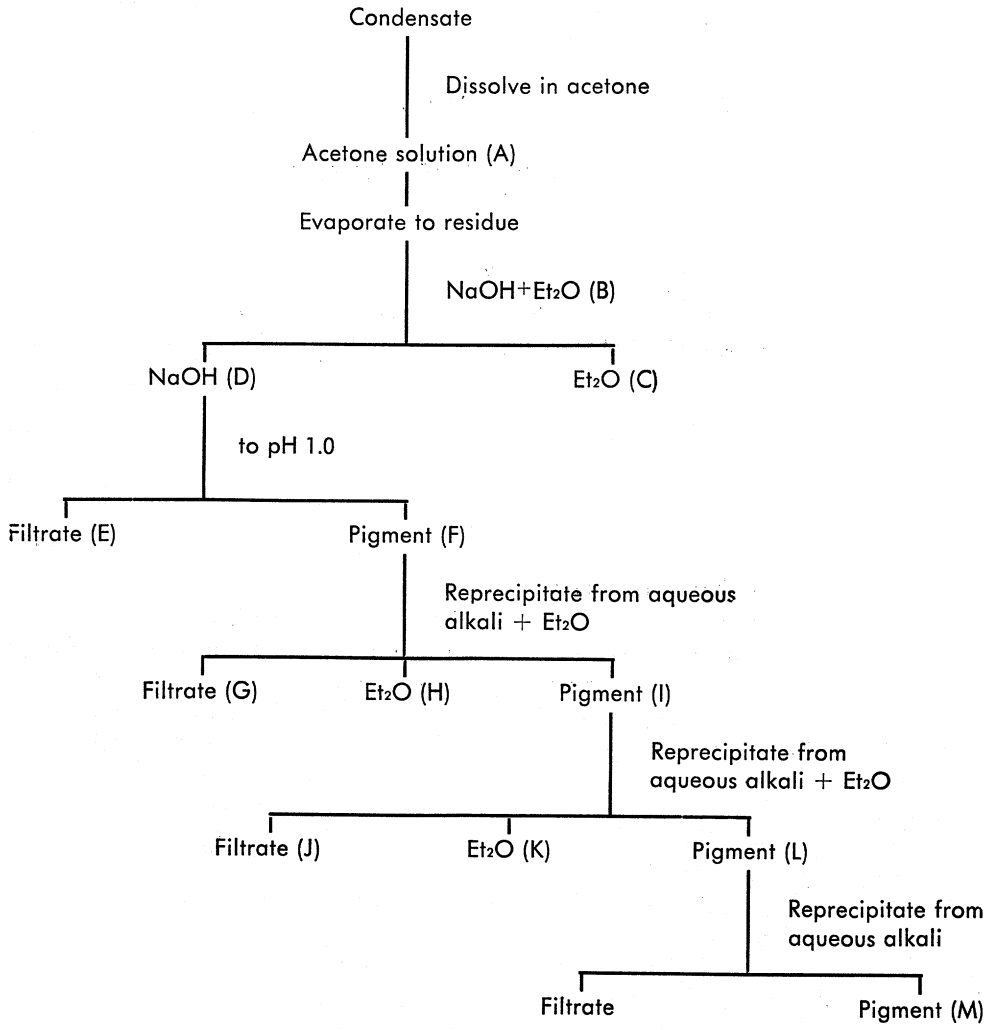
Figure 1 Isolation procedure for acidic pigment from smoke condensate



If these pigments are artifacts produced in the collection apparatus or the isolation procedure, it is conceivable that their smoke precursors may have some degree of tumorigenic activity which escaped detection. The present study is designed to learn more of the origin of pigments in condensate.

METHODS

1. *Cigarettes and Smoking Conditions*

Commercial, 85 mm nonfilter cigarettes were used throughout the study. The cigarettes were smoked mechanically on constant time machines having a capacity of 12—24 cigarettes which were puffed sequentially, using the following conditions: puff volume, 35 ml; puff interval, 2 sec.; and butt length, 20 mm. Usually, 95—100 cigarettes were smoked and the mainstream smoke was collected in 1.0 N sodium hydroxide (300 ml) or in a system having a cold finger and special glass trap (16) immersed in Dry Ice-acetone. The condensate was removed from the latter system with acetone and the solvent was evaporated in vacuo. The acidic pigment was isolated from this residue or from the aqueous alkali traps by precipitation with acid using the previously described method (17) with one modification: extraction of the sodium hydroxide solutions was performed manually with ether (one 10 ml. extraction of the condensate from 95—100 cigarettes) rather than by continuous extraction of the purified pigments with this solvent for 32 hours. In some experiments, the condensate was permitted to remain in the traps or the acetone solution of condensate was stored under different conditions for varying intervals. Sidestream smoke was collected by passage through a scrubber containing aqueous alkali.

2. *Molecular Weight Determination*

Molecular weight determinations were performed using gel filtration or ultracentrifugal analysis. Gel filtrations were performed on polyacrylamide by the methods outlined earlier (4). In the ultracentrifugal analyses, the density gradient method (21) was employed with 5—20% sucrose and phosphate buffer (0.01 M, pH 10.5) as the medium. Sedimentation rates were determined at 50,000 rpm in a SW65 titanium rotor with hemoglobin (mol. wt. 68,000) and cytochrome c (mol. wt. 13,000) as markers. In tracer experiments, pigment was separated by this technic and the contents of the tubes were removed using an apparatus that permitted collection of zones of solution having components of progressively lower molecular weight.

3. Sublimation of Pigments

The nondialyzable, weakly acidic fraction of the condensate pigment (6) and the nondialyzable, chloroform-insoluble fraction of the leaf pigment (6) were sublimed. Approximately 1.0 g of each fraction was heated in a sublimation apparatus at temperatures up to 450° C, using a Woods metal bath. The sublimate was collected and extracted with 1.0 N sodium hydroxide. The alkaline-soluble material that precipitated on addition of acid to pH 1.0 was filtered, washed with water, weighed and analyzed by gel filtration.

4. Dialysis of Pigments

The procedure was essentially similar to the large scale dialysis detailed earlier (4). About 100 mg of pigment were dissolved in 25 ml of 0.01 M phosphate buffer (pH 10.0), the solution was filtered through a high porosity filter and the filtrate was added to a dialysis bag. The bag was dialyzed against the same buffer (100 ml) with two daily changes of buffer. The nondialyzable fraction was removed from the bag, the pH was adjusted to 1.0 and the precipitate was filtered and weighed after drying.

5. Tracer Experiments

A solution of uniformly labelled ^{3}H-nicotine was injected into 15 cigarettes (total, 1.0×10^{10} dpm in 2.43 mg alkaloid) by the previously described technique (19). After equilibration over 72% glycerol ($^{w}/_{w}$), these 15 cigarettes and an additional 85 containing no ^{3}H-nicotine were smoked and the mainstream smoke was collected in acetone-Dry Ice traps. The contents of the traps were fractionated by the above procedure and radioactivity was determined at various steps in the isolation. In other experiments, labelled nicotine was added to the Dry Ice-acetone trap just prior to smoking in amounts equivalent to 19% of that added to the cigarettes. To determine adsorption or occlusion of radioactivity during isolation, 125 mg of acidic pigment and 2.2×10^{8} dpm of tagged nicotine were dissolved in aqueous alkali and the pigment was precipitated several times by the addition of acid to pH 1.0. In the studies utilizing uniformly labelled ^{14}C-sodium acetate, the tagged compound was added to cigarettes (3.1×10^{7} dpm per 100 cigarettes) as the sodium salt or as a mixture of the salt and aqueous HCl designed to yield the free acid. All radioactivity measurements were made using the liquid scintillation technique with a toluene solution of 2,5-diphenyloxazole and 2-p-phenylenebis(5-phenyloxazole) as the scintillators. All measurements were corrected for quenching.

For the saponification of tagged pigment, 20 mg of pigment was added to 10 ml of 2N NaOH. The mixture was saponified under reflux and nitrogen for 4 hours, then cooled to room temperature overnight and finally refluxed an additional 4 hours the next day. The saponification mixture was extracted six times with ether (tot. vol., 100 ml) and the alkaline-insoluble pigment was filtered from the aqueous layer. The alkaline-soluble pigment was precipitated from the filtrate by acidification in the usual manner.

RESULTS AND DISCUSSION

1. Effect of Smoke Collection Systems and Storage Conditions

If the acidic pigment of condensate is partially or entirely formed in the smoke collection system, a progressive increase in isolated pigment weight may occur on extended storage of condensate. Table 1 contains relevant data on the acidic pigment isolated from mainstream smoke condensate under various collection and storage conditions. Condition A refers to a procedure in which the isolation was conducted as rapidly as possible after the mechanical smoking of the cigarettes was completed; the elapsed time for this work was about 1.0 hr. Storage in acetone was studied since the condensate is frequently removed from traps by rinsing with acetone and the acetone extract may be stored for varying periods of time before isolation of the pigment is performed. Although the weight differences in Table 1 are statistically questionable, some trend toward a higher pigment yield on extended storage may be indicated. The variability in these values is greater than that of a previous study in which a modification of the above isolation method gave a coefficient of variation of about 10% (17). This difference may be a reflection of several factors: changes in the composition of the commercial cigarettes during the intervening period, the modification in the isolation procedure, or differences in the characteristics of the smoking machines used in the two investigations.

Table 2 shows the molecular weights of the major subfractions of the acidic pigment from mainstream smoke collected and stored under various conditions. In Condition A, two patterns of distribution were obtained by gel filtration and confirmed by ultracentrifugal analysis. In the majority of cases, the lower molecular weight component predominated. Dialysis of the pigment from one of these runs gave about 30% nondialyzable material. In 2 out of 5 experiments using Condition A, the higher molecular weight component was the major constituent, a pattern similar to that found in earlier work in which stored smoke condensate was the source of the pigment (3, 4). When the condensate was kept in the traps at −79° C for 15 hours or in acetone at room temperature for this period, the predominating component was the high molecular weight subfraction in all

Table 1 Weights and variability of yields of acidic pigment from mainstream smoke condensate collected at -79° C and stored under different conditions

Condition	Storage	No. runs	Yield		
			Aver. (mg)[1]	S. D.	P
A	None	7	79.7	18.3	–
B	15 hrs in trap at -79° C	4	99.2	27.7	0.20
C	15 hrs in acetone at 25° C	4	104.2	34.5	0.17

[1]Per 96 cigarettes. Aver. = average weight from the indicated number of runs. S. D. = standard deviation. P = the probability of statistical difference between A and B or A and C.

Table 2 Molecular weight distributions of subfractions of acidic pigment from smoke collected and stored under various conditions

Condition	Trap	Storage	No. runs	Approx. mol. wt.[1] Gel filt.	Ultracent.
A	Smoke condensed at -79° C	None	3	3,000– 5,000 50,000–100,000	< 13,000 > 68,000
			2	⩾ 100,000 ⩽ 10,000	> 68,000 < 13,000
B	Smoke condensed at -79° C	15 hrs in trap at -79° C	3	⩾ 100,000 ⩽ 5,000	–
C	Smoke condensed at -79° C	15 hrs in acetone at 25° C	3	⩾ 100,000 < 10,000	–
D	Aqueous alkali	None	3	< 3,000 6,000– 8,000 80,000–100,000	–

[1]Gel filt. = gel filtration. Ultracent. = ultracentrifugal analysis. Major component is underlined.

cases. The pigment collected in aqueous alkali gave three groups of components with molecular weights that more closely approximated the majority findings in Condition A.

Sidestream smoke was collected in alkali in a single experiment and two groups of pigments comparable to those obtained from mainstream smoke could be separated in the ultracentrifuge and the proportions of low and high molecular weight constituents more closely approximated the findings in the majority of cases of Condition A.

After several days storage, the filtrates after removal of the precipitated pigments from mainstream smoke condensate slowly developed further amounts of a precipitate. Ultracentrifugal analysis of this material showed it to consist entirely of the low molecular weight component.

These results show that two groups of components, one of high and one of low molecular weight, are present in the acidic pigments from condensates of mainstream smoke collected and stored under different conditions. The proportions of the two groups seem to vary with storage time although in some cases, no great difference can be found in pigments isolated immediately or after storage. The findings with the freshly prepared pigments from acetone-Dry Ice traps or from aqueous alkali traps may indicate that some polymerization of the low molecular weight component (about 3,000–8,000) to the high molecular weight component (about ⩾ 100,000) is occurring on storage.

2. *Sublimation of Pigments*

Considering the physical properties of the leaf and condensate pigments, only two mechanisms for transference of the pigment from leaf to smoke would seem possible: cellular disruption or sublimation. To determine the latter, major subfractions of the leaf and condensate pigments were sublimed. The findings are summarized in Table 3. At 760 mm, which roughly approximates the pressure existing during the smoking process, neither pigment sublimed at temperatures up to 450° C. At 0.8 mm and 350°–360° C, considerable gas was evolved and a substantial portion of each pigment sublimed although only a small amount of the sublimate was soluble in aqueous alkali. The results shown in Table 3 were obtained with the alkaline-soluble material in the sublimates. The lower molecular weight component in each case was in the same general range as the comparable component in the freshly prepared or stored acidic pigment from condensate or smoke collected in aqueous alkali. The other component in the sublimates appeared to be lower in molecular weight than its counterpart in the condensate pigment. The relatively small differences in the thermal behavior of the leaf and condensate pigments are in agreement with some aspects of recent work on the thermal decomposition of the two substances (22).

Although obtained under entirely different conditions than exist in the smoking process, the patterns of molecular weights of the sublimates are superficially similar to those found in the condensate pigment. Thus, the possibility cannot be excluded that the leaf pigment may sublime with or without extensive structural degradation during the smoking process.

3. *Tracer Studies*

Both leaf and smoke pigments contain nicotine that is released by alkaline fusion (5, 10, 18) and the levels of the alkaloid are in the same general range for both

Table 3 Yields and molecular weights of sublimates and fractions thereof obtained from acidic condensate and leaf pigments

Origin	T (° C)	Pressure (mm)	Sublimate Yield (%)	Sublimate Mol. wt.[1]
Leaf	350–360	0.8	21.7	7,000 20,000
	450	760	0	–
Smoke	350–360	0.8	22.0	5,000 19,000
	450	760	0	–

[1]By gel filtration. Major component is underlined.

Table 4 Distribution of radioactivity in isolation procedure for condensate pigment when ^{3}H-nicotine is added to cigarettes, collection trap or Fraction D in Figure 1

Fraction	^{3}H-Nicotine added to								
	Cigarettes[1]			Trap[2]			Fraction D[3]		
	dpm	Pigment wt (mg)	Specific activity	dpm	Pigment wt (mg)	Specific activity	dpm	Pigment wt (mg)	Specific activity
A	—	—	—	1.9×10^9	—	—	—	—	—
C	1.0×10^9	—	—	1.4×10^9	—	—	—	—	—
D	4.0×10^8	—	—	4.5×10^8	—	—	2.2×10^8	—	—
E	3.3×10^6	—	—	2.2×10^8	—	—	1.8×10^8	—	—
F	5.1×10^7	—	—	2.4×10^6	136	17,600	7.8×10^5	97	8,000
G	1.8×10^7	—	—	—	—	—	—	—	—
H	3.8×10^5	—	—	—	—	—	—	—	—
I	7.6×10^5	78	9,700	1.4×10^6	63.7	22,000	3.8×10^5	58	6,600
J	3.1×10^6	—	—	—	—	—	—	—	—
K	9.0×10^3	—	—	—	—	—	—	—	—
L	5.0×10^5	—	—	1.2×10^6	48.6	24,700	3.0×10^5	48	6,300
M	4.3×10^5	30	14,300	7.8×10^5	31.2	25,000	—	—	—

[1] 1.0×10^{10} dpm added to cigarettes.
[2] 1.9×10^9 dpm added to the trap.
[3] 125 mg pigment + 2.2×10^8 dpm of tagged alkaloid added at isolation step comparable to Fraction D.

pigments (10). However, only the condensate pigment contains nicotine that can be released by saponification (6). The saponifiable nicotine may arise by two possible routes. If the pigment is a partial or entire artifact, the precursors may react with nicotine in the collection traps forming a saponifiable linkage; if the pigment is formed completely in smoke, the precursor will react with the nicotine in the smoke to yield a similar linkage. These possibilities can be differentiated by adding ^{3}H-nicotine to either cigarettes or the collection system and studying the radioactivity in the isolated pigment from the condensates. To eliminate the possibility that radioactivity is acquired by the pigment through adsorption or occlusion during isolation, a third condition is necessary in which ^{3}H-nicotine is added at step D in Figure 1.

Table 4 presents data on such experiments. About 14% of the radioactivity added to the cigarette was transferred to the mainstream smoke, which confirms previous data (20). A relatively high proportion of radioactivity was found in Fraction D. This may have been due to incomplete extraction with ether or the presence of oxidation products of nicotine in the tagged material. Most of the oxidation products of nicotine are bases (1) and would be expected to react in a manner similar to nicotine in reactions with the pigment. Also, the latter is known to contain oxidation products of nicotine (1, 6). Thus, the presence of such oxidation products should not obviate the purpose of the experiment. As shown in Table 4, significant radioactivity was obtained in the isolated pigments under all three experimental conditions; however, the percentage of available radioactivity taken up by the pigments was extremely small. These findings show that the pigment radioactivity may be due to several factors, including physical occlusion, adsorption and reaction during isolation, collection and smoke genesis.

To confirm that physical occlusion was not entirely responsible for the radioactivity in the isolated pigment, a portion of the pigment obtained when ^{3}H-nicotine was added to cigarettes was separated by the ultracentrifugal technique. The solution containing the separated material was then removed from the tube in a special apparatus that permits zones of solution containing substances of decreasing molecular weight to be successively drawn off. The findings indicated that the radioactivity levels paralleled the concentrations of high molecular weight subfractions of the pigment. Since the pigment was in alkaline solution during the separation, no opportunity for occlusion of nicotine existed in this experiment and any tagged nicotine present as the unionized base would have appeared exclusively in the uppermost layer of the tube. This confirmed that nicotine is actually incorporated into the pigment molecule and not simply occluded or adsorbed during isolation. The incorporation may occur during the isolation as well as in the trap or during smoke genesis.

Although radioactivity was incorporated into the condensate pigment when the tagged alkaloid was added to either cigarettes or the collection system, it was possible that the nicotine in each case still arose from two sources: incorporation into the precursor during smoke formation and subsequent reaction during collection system and/or isolation. If the linkages differed in these two cases, the amounts of nicotine released on saponification of the pigments might also differ. Table 5 presents data on the distribution of radioactivity on saponification of pigments produced when tagged alkaloid was either added to the cigarettes or to the collection system. In each case the pigments were saponified and all products were collected after fractionation by a procedure superficially similar to Figure 1. The greatest difference in the products from the two pigments was in the proportions of pigment that became insoluble in alkali after saponification. The levels of radioactivity in the ether-soluble fractions and acidified filtrates were essentially similar for both pigments. Thus, it appears that little difference

Table 5 Distribution of radioactivity in saponification products from condensate pigments isolated when ^{3}H-nicotine was added to cigarettes or the collection trap

Fraction[1]	^{3}H-Nicotine added to			
	Cigarettes		Trap	
	Total dpm	Distribution (%)	Total dpm	Distribution (%)
Ether solubles (C)	1.7×10^4	7.4	2.3×10^4	7.8
Filtrate (E)	1.4×10^5	60.9	1.8×10^5	61.0
Insoluble pigment[2]	2.1×10^4	9.1	4.8×10^4	16.3
Soluble pigment (F)	5.2×10^4	22.6	4.4×10^4	14.9

[1]Analogous fractions in Figure 1 are shown in parentheses.
[2]Insoluble material formed in the saponification.

is observed in the manner in which nicotine is linked in the pigments under the two experimental conditions.

All of these findings show that very small amounts of nicotine can be acquired as a pigment moiety through reactions with nicotine in smoke, in the collection system and/or during isolation. The nicotine acquired from these sources represents a very small percentage of the total nicotine and bases released from the pigment by alkaline fusion or saponification. Based on the level of nicotine found in the saponification products of the condensate pigment in previous work (about 0.086% of total pigment weight) (6), the nicotine acquired by vapor phase reactions in smoke and/or subsequent artifact reaction is no more than 3% of the total nicotine therein. This would indicate that most of the nicotine in the condensate pigment is probably derived from leaf precursors, such as the closely related leaf pigment.

In contrast to nicotine, both leaf and condensate pigments contain volatile fatty acids that can be released by saponification (6). If cigarette tobacco (1.0 g per cigarette) contains about 2% leaf pigment (7) and the mainstream smoke condensate (25 mg per cigarette) therefrom contains about 5% pigment (17), the percentage transference of pigment from leaf to smoke is about 6%, assuming the leaf pigment is the precursor. Using the previously published values for the percentage of total saponifiables and yields of C_1 and C_2 acids in the two pigments (6), the total hydrolyzable C_1 and C_2 acids in the leaf and condensate pigments from one cigarette and the smoke therefrom are 0.95 mg and 0.011 mg, respectively. Thus, uptake of C_1 and C_2 acids during genesis of the condensate pigment would not be expected; in fact, some loss of these acids would be anticipated.

Experiments to determine this point were conducted by smoking cigarettes containing ^{14}C-labelled sodium acetate or acetic acid. No uptake of radioactivity by the condensate pigment was observed with either the salt or free acid when 3.1×10^7 dpm were added to 100 cigarettes. Likewise, condensate pigment did not become radioactive when the tagged sodium acetate was added at Step A in the isolation procedure and the pigment was isolated after 2 days storage of the acetone solution at room temperature. The bulk of the radioactivity in the isolated fractions was found in Filtrate E in all of these experiments. Gas chromatographic analysis of an ether extract of Filtrate E showed that essentially all of the radioactivity was due to unchanged acetic acid which had distilled over in the smoke when the tagged compounds were added to the cigarettes. These findings indicate that acetic acid does not participate in artifact reactions leading to synthesis of pigment in the collection system or isolation procedure. The results do not eliminate the possibility that a low molecular weight precursor containing acetic acid polymerizes or otherwise reacts with other components in the traps to yield the isolated pigment. On the other hand, if the precursors and components exhibit such a high degree of reactivity for one another, it is just as possible that they would react in the particulate matter of smoke as well as in the traps.

SUMMARY

The yields of acidic brown pigment isolated from cigarette smoke collected and stored under various conditions show no significant differences; however, some tendency may exist for a small increase in yield on extended storage of the condensate in traps at −79° C or in acetone solution at room temperature. The molecular weights of subfractions of the pigment are variable when the pigment is immediately isolated from freshly prepared condensate. Storage of condensate results in a higher proportion of high molecular weight subfraction in the pigment in all cases. Both leaf and condensate pigments sublime at high temperature and low pressure yielding a sublimate that contains some subfractions with molecular weights of the same general magnitude as those in the isolated condensate pigment. Nicotine may be acquired as a moiety in the condensate pigment through artifact reactions in the collection system or during the isolation. However, the nicotine acquired in this way apparently represents a very small amount of the total saponifiable nicotine in the pigment. The acetic acid moiety of the condensate pigment is not acquired by such artifact reactions. Although part of the condensate pigment structure may be formed through reactions in the collection system or during isolation, no evidence was obtained that the condensate pigment is largely an artifact.

ZUSAMMENFASSUNG

Die Ausbeuten an sauren braunen Pigmenten, die aus Cigarettenrauch isoliert wurden, zeigten in Abhängigkeit von der Niederschlagung des Rauches und der Lagerung der Kondensate keine signifikanten Unterschiede. Allerdings könnte eine Tendenz für einen schwachen Ausbeuteanstieg nach ausgedehnter Lagerung des Kondensates in Fallen bei −79° C oder in Aceton gelöst bei Raumtemperatur bestehen. Die Molgewichte von Unterfraktionen des Pigments sind unterschiedlich, wenn das Pigment sofort aus frisch gewonnenem Kondensat isoliert ist. Lagerung von Kondensat führt in jedem Fall zu einem größeren Anteil an hochmolekularen Unterfraktionen im Pigment. Sowohl Blatt- als Kondensatpigmente sublimieren bei hoher Temperatur und niedrigem Druck zu einem Sublimat, das einige Unterfraktionen mit Molgewichten derselben allgemeinen Größe enthält wie die in dem isolierten Kondensatpigment. Nikotin kann als Bestandteil in das Pigment eventuell durch nachträgliche Reaktionen im Sammelsystem oder während der Isolierung gelangen. Das auf diesem Wege aufgenommene Nikotin repräsentiert jedoch anscheinend nur einen sehr kleinen Teil des gesamten durch Verseifung freisetzbaren Nikotins im Pigment. Der Essigsäureanteil des Kondensatpigments ist nicht durch solche nachträglichen Reaktionen entstanden. Obgleich Teile der Struktur des Kondensatpigments durch Reaktionen im Sammelsystem oder während der Isolierung entstanden sein könnten, wurden keine Beweise erhalten, daß das Kondensatpigment weitgehend ein Artefakt ist.

RÉSUMÉ

Le rendement en pigment brun acide extrait de la fumée de cigarette ne varie pas significativement en fonction des conditions de collecte et de conservation du condensat; toutefois il pourrait y avoir une tendance à un léger accroissement du rendement en cas de conservation prolongée du condensat dans des pièges à −79° C, ou en solution acétonique à la température ambiante. Les poids moléculaires des sous-fractions du pigment sont variables quand le pigment est immédiatement isolé d'un condensat fraîchement préparé. Dans tous les cas, le stockage du condensat conduit à une proportion plus élevée de la sous-fraction du pigment à poids moléculaire élevé. Les pigments de la feuille et celui du condensat se subliment à température élevée et faible pression, donnant un sublime qui contient des sous-fractions dont les poids moléculaires sont du même ordre que ceux des sous-fractions du condensat isolé du pigment. On peut obtenir de la nicotine paraissant être une portion du pigment du condensat mais c'est un artefact provenant de réactions dans le système de collecte ou au cours de l'isolement. Toutefois la nicotine obtenue de cette façon ne représente qu'une très petite quantité de la nicotine totale saponifiable dans le pigment. La portion acide acétique du condensat du pigment n'est pas un artefact. Bien qu'une partie de la structure du pigment du condensat puisse être formée par l'intermédiaire de réactions dans le système de collecte ou au cours de l'isolement, on n'a pas obtenu de preuve que le pigment du condensat soit essentiellement un artefact.

REFERENCES

1. Stedman, R. L.: Chem. Rev. 68 (1968) 153.
2. Swain, A. P., Cooper, J. E., and Stedman, R. L.: Cancer Res. 29 (1969) 579.
3. Stedman, R. L., Chamberlain, W. J., and Miller, R. L.: Chemistry & Industry (1966) 1560.
4. Dymicky, M., and Stedman, R. L.: Phytochem. 6 (1967) 1025.
5. Dymicky, M., and Stedman, R. L.: Tobacco Sci. 12 (1968) 7.
6. Dymicky, M., and Stedman, R. L.: Tobacco Sci. 13 (1969) 45.
7. Chortyk, O. T.: Tobacco Sci. 11 (1967) 137.
8. Wright, H. E., Jr., Burton, W. W., and Berry, R. C., Jr.: Arch. Biochem. Biophys. 86 (1960) 94.
9. Wright, H. E., Jr., Burton, W. W., and Berry, R. C., Jr.: Phytochem. 3 (1964) 525.
10. Dymicky, M., Chortyk, O. T., and Stedman, R. L.: Tobacco Sci. 11 (1967) 42.
11. Kallianos, A. G., Shelburne, F. A., Means, R. E., Stevens, R. K., Lax, R. E., and Mold, J. D.: Biochem. J. 87 (1963) 596.
12. Neurath, G., Pirmann, B., Lüttich, W., and Wichern, H.: Beitr. Tabakforsch. 3 (1965) 251.
13. Kallianos, A. G., Means, R. E., and Mold, J. D.: Tobacco Sci. 12 (1968) 125.
14. Nall, J. F.: 20th Tobacco Chemists' Res. Conf., Winston-Salem, N. C., 1966.
15. Bock, F. G., Swain, A. P., and Stedman, R. L.: Cancer Res. 29 (1969) 584.
16. Schepartz, A. I.: Tobacco Sci. 3 (1959) 144.
17. Chamberlain, W. J., and Stedman, R. L.: Tobacco Sci. 10 (1966) 162.
18. Dymicky, M., and Stedman, R. L.: Tobacco Sci. 12 (1968) 75.
19. Stedman, R. L., Lakritz, L., and Strange, E. D.: Beitr. Tabakforsch. 5 (1969) 13.
20. Harlan, W. R., and Moseley, J. M.: Encyclopedia of Chemical Technology, Vol. 14, p. 242, The Interscience Encyclopedia, Inc., New York, N. Y., 1955.
21. Britten, R. J., and Roberts, R. B.: Science 131 (1960) 32.
22. Burdick, D., and Burton, H. R.: Tobacco Sci. 13 (1969) 16.

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